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Labelling of a pyrazole derivative with ^{131}I and investigation of its radiopharmaceutical potential

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We investigated the radiolabeling efficiency, *in vitro* stability, and biodistribution of radioactive iodine (^{131}I)-labeled 4-benzoyl-1-(4-carboxyphenyl)-5-phenyl-1*H*-pyrazole-3-carboxylic acid (P3CA). A quality-control study of the labeled substance (^{131}I-P3CA) was conducted using electrophoresis and radio thin-layer chromatography (RTLC). Biodistribution studies were undertaken in 9 female albino Wistar rats. Rats were killed at various times (15, 60 and 180 min), their organs removed, and percentage of injected dose per gram (ID%/g) values calculated. The labeling yield of P3CA was 97.08%±0.80%. The maximum uptake of ^{131}I-P3CA was seen in the lungs, stomach and spleen at 15 min. The uptake of labeled compound decreased over time in the lungs, whereas that in the stomach decreased. These data suggest that ^{131}I-P3CA had high binding efficiency, high uptake in the lung, and sufficient stability to be used in diagnostic studies.

pyrazole derivative, lungs, ^{131}I, biodistribution, rat

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Radiopharmaceuticals have been used in nuclear medicine as tracers in diagnosing and treating several diseases. Radionuclides and radiopharmaceuticals are used widely for different purposes. Radioactive iodine (^{131}I) is used routinely in nuclear medicine. Chemotherapy with ^{131}I (first introduced in 1946 for the treatment of thyroid cancer) is the most efficacious method for treating hyperthyroidism and thyroid cancer.

Pyrazole derivatives are a class of heterocyclic compound containing 1,2 diazole systems [1]. They are important substances and have gained widespread attention due to their biological activities. Several studies have revealed that pyrazoles have important properties: antimicrobial [2,3] analgesic [4,5], antiviral [6,7], antitumor [8,9], anti-inflammatory [10,11], cytotoxic [12], antihistaminic [13], pesticidal [14], antifungal [15,16], anticonvulsant [17] and antidepressant [18].

Several studies have investigated the synthesis and characterization of different pyrazole-containing ring systems endowed with potential chemotherapeutic activities [19–21]. Pyrazoles are known to exert anticancer effects by inhibiting enzymes that have important roles in cell division [22]. The Biological Committee of the National Cancer Institute has selected three pyrazole compounds to optimize the exposure time to these agents and to define their possible mechanisms of action using *in vivo* studies [23].

The antibacterial activity of 4-benzoyl-1-(4-carboxyphenyl)-5-phenyl-1*H*-pyrazole-3-carboxylic acid (P3CA) affects Gram-positive and Gram-negative bacteria [3]. The biodistribution of P3CA has not been investigated. The purpose of the present study was the labeling of P3CA with ^{131}I and to determine the radiopharmaceutical potential of the labeled compound.

1 Materials and methods

All reagents were: available commercially; of analytical grade;

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used without further purification. 4-benzoyl-1-(4-carboxyphenyl)-5-phenyl-1H-pyrazole-3-carboxylic acid was provided by the Organic Chemistry Division, Yuzuncu Yil University (Van, Turkey). 131I was obtained from the Department of Nuclear Medicine, Ege University (Izmir, Turkey).

1.1 Labeling procedure

(1) Synthesis of inactive I-P3CA. Inactive I-P3CA was synthesized according to the method of Akhlaghinia and Rahmanib, with slight modifications [24]. P3CA (20.0 mg, 0.05 mmol), iodogen (21.2 mg, 0.05 mmol), SiO_2 (25.0 mg, 0.42 mmol), and iodine (12.4 mg, 0.05 mmol) were dissolved in 5.0 mL methane dichloride. Next, we added 3.0 mL methanol and 25.0 mL distilled water to the mixture and stirred it for 30 min at room temperature. Sodium thiosulfide (10%) was added over the solution until the dark-orange color disappeared. The progress of the reaction was monitored by thin-layer chromatography (TLC). When the reaction was complete, the solvent was removed by vacuum, and the residue washed with methane dichloride (5.0 mL). The precipitate was dissolved in methanol to separate the impurities, and the solvent removed by vacuum. The residue was dried over anhydrous $MgSO_4$ to provide inactive I-P3CA.

(2) Synthesis of ^{131}I-P3CA. ^{131}I-P3CA was synthesized according to the method of Mertens [25]. A solution of I-P3CA (5.0 mg in 20.0 μL DMSO) was mixed with a solution of ascorbic acid (2.0 mg in 200.0 μL distilled water). Next, 10.0 μL $CuSO_4$ (1.0 mg $CuSO_4$/40.0 mL distilled water) and 400.0 μL (10 mCi/mL) $Na^{131}I$ were added to the solution and mixed for 2 h at 100°C. The solution was allowed to stand at room temperature for 12 h to obtain ^{131}I-P3CA.

(3) Oxidation of ^{131}I. An iodogen (1.0 mg)-coated eppendorf tube was charged with 500 μCi of $Na^{131}I$. The reaction mixture was allowed to stand at room temperature for 25 min without stirring. After 25 min, quality controls were checked. During the labeling of ^{131}I-P3CA, there are three types of radioactive iodine. The first one is oxidized and connected to P3CA. The second one is oxidized but does not react with P3CA. The third one is neither oxidized nor does it react with P3CA. The oxidation of ^{131}I was conducted to find unreacted oxidized ^{131}I.

1.2 Quality controls

The following quality-control studies were done with radio thin-layer chromatography (RTLC) and paper electrophoresis to confirm the labeling efficiency of ^{131}I-P3CA.

(1) RTLC procedure. Each RTLC cellulose-coated plate was covered with a cello-band after its development and cut into widths of 0.5 cm. Various solvent mixtures were used as developers [(solvent A: acetic acid, water, *n*-butanol; 1/2/4, *v*/*v*/*v*), (solvent B: water, DMSO, ethyl alcohol; 1/2/5/, *v*/*v*/*v*), (solvent C: distilled water)]. The developed sheet was dried and cut into widths of 0.5 cm. Radioactivity on the plates was measured using a Cd(Te) detector equipped with a RAD 501 single-channel analyzer. RTLC figures were obtained by plotting counts *versus* distance. R_f values and labeling yields were derived from these chromatograms.

(2) Paper electrophoresis. Electrophoresis was achieved with a Gelman Electrophoresis Chamber, model 51170. The cathode and anode poles and application points were indicated on cellulose strips moistened by Physiologic Serum Solution (0.9% NaCl). After the samples were set onto the strips, they were placed in the electrophoresis chamber. During 2 h, a voltage of 300 V was applied. Developed strips were dried and cut into 1-cm pieces. The amount of radioactivity in each sample was quantified using a radiation monitor, RAD-501, single-channel analyzer.

1.3 Lipophilicity

A 100.0-μL aliquot of the labeled sample was mixed with 3 mL of 1-octanol and 3 mL of water in a test tube. The tube was vortex-mixed (3×1 min), incubated for 1 h at room temperature, and centrifuged at 3000 r/min for 5 min. Then, 0.5-mL aliquots were taken from each phase for counting. The partition coefficient=log*P* (counts in *n*-octanol layer/counts in aqueous layer).

1.4 Serum and physiological serum stability of ^{131}I-P3CA

^{131}I-P3CA (300.0 μL) was incubated with 600.0 μL of fresh human and physiological serum at 37°C for 4 h. During incubation, labeled substances were examined at 15 min and 1, 3 and 24 h by RTLC.

1.5 Biodistribution studies in rats

Animal experiments were conducted using protocols approved by the Animal Care and Use Committee of Afyon Kocatepe University (Afyonkarahisar, Turkey). The experiments involved female Albino Wistar rats (≈100–140 g). Three rats were used for each set of experiments. To prevent iodine uptake by the thyroid gland, 10.0 mg of potassium iodide was added to 1.0 L of the drinking water consumed by the rats. The pH of the ^{131}I-labeled compound was adjusted to 7.0 with 1.0% NaOH solution. It was sterilized by passing through a 0.22-μm membrane filter.

The compound was injected into the tail vein of rats (5.0 mg ^{131}I -P3CA per rat). Rats were killed in an ether atmosphere after certain times (15, 60, or 180 min) and their organs removed. All collected tissues were weighed and the radioactivity of ^{131}I -P3CA calculated as the percentage of injected dose per gram (ID%/g).

1.6 Statistical analyses

Data are the mean±SD for parametric variables. Parametric

variables were compared using Pearson's correlation and one-way analysis of variance with *post hoc* analyses using the Duncan test. $P<0.05$ were considered significant.

2 Results and discussion

The labeled compound is shown in Figure 1. High radiolabeling efficiency and good stability of radiolabeled substances are essential for successful immunoconjugate imaging or therapy. The labeling yield of ^{131}I-P3CA was 97.08%±0.80%. Electrophoresis revealed ^{131}I-P3CA to be neutral (Figure 2) because it did not move toward either electrode.

Lipophilic substances are soluble in fat. The partition coefficient [LogP (counts in n-octanol layer/counts in aqueous layer)] of ^{131}I-P3CA was –0.43 (n=3) at pH 7.0. The theoretical lipophilic value of ^{131}I-P3CA was calculated using the ACD/Labs program and was 5.75.

We believe that copper forms a complex with I-P3CA because it contains nitrogen and oxygen atoms. Therefore, we added $CuSO_4$ during the labeling of P3CA with ^{131}I. According to the theoretical value, I-P3CA is more soluble in the octanol phase (log$P$=5.75) than the water phase. However, the complex of ^{131}I-P3CA with copper is more soluble in the water phase; the solubility changes with complex formation. Hence, the lipophilic value is also decreased.

The R_f values for the radioactive compounds are given in Table 1. The radiolabeled compound (^{131}I-P3CA) did not move in any solvent media, but the other radioactive substances moved from their initial starting point.

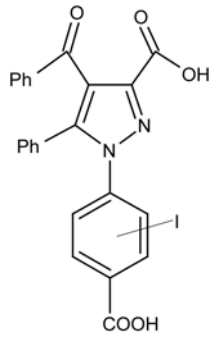

Figure 1 Structure of the labeled compound (^{131}I-P3CA).

In vitro stability results showed that ^{131}I-P3CA deiodinates with time. The labeling percentages were 97.08%±0.80%, 97.05%±2.41%, 97.02%±2.24%, 97.01%±3.28%, and 96.92%±4.11% at 15, 30, 60, and 180 min and 24 h, respectively (Table 2). The stability decreased very slowly over time. According to the serum stability data, the radiolabeling yield was 97.08%±0.80%, 95.56%±3.39%, 95.01%±3.83%, 91.43%±4.18%, and 86.46%±9.45% at 15, 30, 60, and 180 min and 24 h, respectively (Table 2). These results suggest that the radiolabeled compound is suitable for imaging. Decreases in serum stability and uptake of physiological serum did not show similar trends across the same time period. Serum contains different types of substances and metals ions that might affect the structure of ^{131}I-P3CA. Hence, the deterioration of ^{131}I-P3CA in serum is faster than in physiological serum.

The biodistribution of ^{131}I-P3CA to all studied organs is given (Table 3) in %ID/g. A high uptake of ^{131}I-P3CA was observed in the lungs (ID%/g=42.98) at 15 min. This uptake of the radio-substance decreased over time (ID%/g=9.65 and 3.26 at 60 and 180 min, respectively). The other important organ for ^{131}I-P3CA uptake was the stomach. The uptake of ^{131}I-P3CA increased over time (ID%/g 3.30, 3.54 and 6.37 at 15, 60, and 180 min, respectively) in the stomach. The clearance of ^{131}I-P3CA from the blood (2.78, 0.82 and 0.46 at 15, 60 and 180 min, respectively) changed sharply. A significant amount of ^{131}I-P3CA was absorbed in the spleen. The initial uptake of ^{131}I-P3CA was high, then decreased and then increased again in the heart, liver, thyroid, kidneys, spleen, muscle, intestine, ovaries, pancreas, head, spinal cord, and uterus.

There were significant positive correlations between some of the organs (Table 4).

Effective radiolabeling of P3CA with ^{131}I required the adoption of the normal heating method. While labeling P3CA with ^{131}I, 14 h elapsed. The reaction time was higher than that required for radiolabeling an imidazole derivative (^{131}IAZGP) using the ^{131}I–I exchange reaction [26].

Radiopharmaceuticals can, in general, be categorized on the basis of their unique mechanism of localization in a

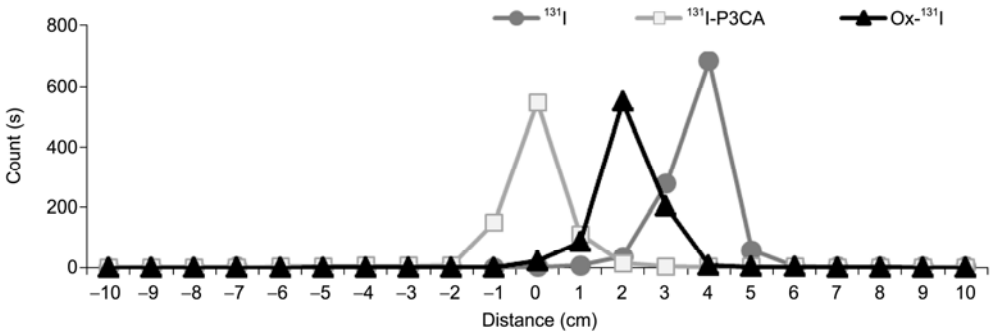

Figure 2 Electrophoretogram of ^{131}I$^-$, oxidized-^{131}I and ^{131}I-P3CA.

Table 1 R_f values of radioactive components in RTLC [a)]

Solvent	^{131}I	Ox-^{131}I	^{131}I-P3CA
A	0.25	0.00	0.00 (application point)
B	1.00	1.00	0.00
C	0.88	1.00	0.00

a) Ox-^{131}I: oxidation of iodine (^{131}I); solvent A: *n*-butanol, water, acetic acid (4/2/1, v/v/v); solvent B: DMSO, ethyl alcohol, water (2/5/1, v/v/v); solvent C: distilled water.

Table 2 Stability of ^{131}I-P3CA in physiological serum and human serum

Time (h)	Yield (%) in physiological serum	Yield (%) in human serum
0	97.08±0.80	97.08±0.80
1	97.05±2.41	95.56±3.39
2	97.02±2.24	95.01±3.83
3	97.01±3.28	91.43±4.18
4	96.92±4.11	86.46±9.45

Table 3 Percentage of giving dose per gram (ID%/g) ^{131}I-P3CA in normal rat tissues 15, 60 and 180 min post-injection

Organ	15 min		60 min		180 min	
	Mean	SD	Mean	SD	Mean	SD
Heart	0.74	0.14	0.28	0.14	0.91	0.82
Lung	42.98	1.94	9.65	2.13	3.26	1.24
Liver	3.63	2.28	1.89	0.83	3.82	2.18
Kidneys	1.01	0.05	0.39	0.17	0.67	0.13
Small intestine	1.24	0.73	0.44	0.31	1.11	0.35
Large intestine	0.72	0.46	1.00	0.69	0.41	0.16
Stomach	3.30	1.81	3.54	2.44	6.37	1.49
Spleen	2.10	1.13	0.74	0.25	2.08	0.90
Pancreas	1.14	0.46	0.49	0.26	0.57	0.20
Blood	2.78	1.45	0.82	0.31	0.46	0.27
Muscle	0.69	0.50	0.28	0.30	0.43	0.11
Ovary	1.45	1.13	0.20	0.13	0.59	0.22
Thyroid	0.56	0.19	0.27	0.09	0.65	0.15
Brain	0.23	0.19	0.05	0.04	0.12	0.07
Spinal cord	0.36	0.33	0.21	0.20	0.44	0.22
Uterus	1.35	1.05	0.30	0.20	0.73	0.17
Urinary bladder	2.29	1.59	2.24	2.38	4.02	1.67

Table 4 Positive correlations between the organs of ^{131}I-P3CA

Organs	*r*	*P*	Organs	*r*	*P*
Lung-blood	0.85	<0.0030	Pancreas-blood	0.87	<0.0017
Liver-spleen	0.96	<0.0002	Pancreas-ovary	0.88	<0.0013
Liver-thyroid	0.85	<0.0030	Pancreas-brain	0.85	<0.0034
Liver-spinal cord	0.94	<0.0005	Pancreas-uterus	0.85	<0.0031
Small intestine-spleen	0.90	<0.0007	Blood-ovary	0.91	<0.0005
Small intestine-ovary	0.86	<0.0024	Blood-brain	0.85	<0.0034
Small intestine-thyroid	0.91	<0.0005	Muscle-ovary	0.86	<0.0023
Small intestine-brain	0.85	<0.0033	Muscle-brain	0.85	<0.0036
Small intestine-spinal cord	0.86	<0.0029	Muscle-uterus	0.91	<0.0006
Small intestine-uterus	0.89	<0.0010	Ovary-brain	0.97	<0.0001
Spleen-brain	0.87	<0.0001	Ovary-uterus	0.98	<0.0001
Spleen-spinal cord	0.90	<0.0009	Brain-uterus	0.93	<0.0002

specific tissue/organ. Radiolabeled substances should be within an appropriate size range for specific organ uptake or biodistribution. For example, uptake in lungs is high for particles larger than 10 μm [27], so ^{131}I-P3CA uptake in the lungs was high. One possible explanation for this finding is that ^{131}I-P3CA forms a more complicated complex with copper, and this complicated complex may be retained in lung capillaries.

Imidazole derivatives are labeled with different radionuclides: ^{99m}Tc, ^{64}Cu, ^{131}I, and ^{18}F. For an imidazole derivative to connect to a radionuclide, a substance that connects to the imidazole derivative and the radionuclide is necessary. These substances should contain an aromatic group for nucleophilic substitution with the radioactive halogen group or nitrogen, oxygen, phosphorus, and sulfur atoms to create compounds with radioactive metals. During the labeling of I-P3CA with ^{131}I, I-P3CA could have formed a complex with copper, and undergone nucleophilic substitution with ^{131}I.

^{64}Cu-labeled two monoclonal antibodies (DOTA-cT84.66 and DOTA-cBR96, DOTA=1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) have high uptake in the lungs at 15 min, but their uptake decreases over time in the lungs [28]. Similar results were obtained in the present study. The maximum uptake values for the radioiodinated thymidine analog (^{125}I-IUdR) [29] in lung metastases in mice and radiolabeled IAZGP [30] in the lungs were ≈0.6 (ID%/g), but uptake of ^{131}I-P3CA in the lung was more than that stated previously.

Comparing the biodistribution of ^{131}I-P3CA with that of ^{64}Cu-NOTA-TRC105 and ^{64}Cu-DOTA-TRC105 ≈4 h after injection, ^{131}I-P3CA radioactivity in the blood was significantly lower than that of ^{64}Cu-DOTA-TRC105 and ^{64}Cu-NOTA-TRC105. According to Zing at al., who reported on the biodistribution of ^{64}Cu-labeled monoclonal antibody with NOTA and DOTA, the clearance of labeled molecules in the blood was considerably slower than what we observed with ^{131}I-P3CA. Some of the processes that metabolize and excrete drugs in the liver and kidneys [31] absorbed a considerable part of ^{131}I-P3CA soon after injection into the liver and kidneys. The maximum uptake of ^{131}I-P3CA occurred at 180 min in the liver and at 15 min in the kidneys.

The uptake of ^{131}I-P3CA in the brain was not significant. This result was expected because the molecular weight of the ^{131}I-P3CA complex with copper is >500 Da. If drugs have a low molecular mass (<400–500 Da) and high lipid solubility, they can pass through the blood-brain barrier readily [32].

3 Conclusion

The original goal of the present study was to calculate the biodistribution of ^{131}I-P3CA in rats. Additionally, we showed that the labeling efficiency of ^{131}I-P3CA was high and that ^{131}I-P3CA accumulation in the lungs was appreciable. We feel that ^{131}I-P3CA has sufficient stability to be used in diagnostic studies. If P3CA is labeled with ^{123}I or ^{64}Cu instead of ^{131}I, it could prove to be a radiopharmaceutical target for lung imaging in single-photon emission computed tomography.

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